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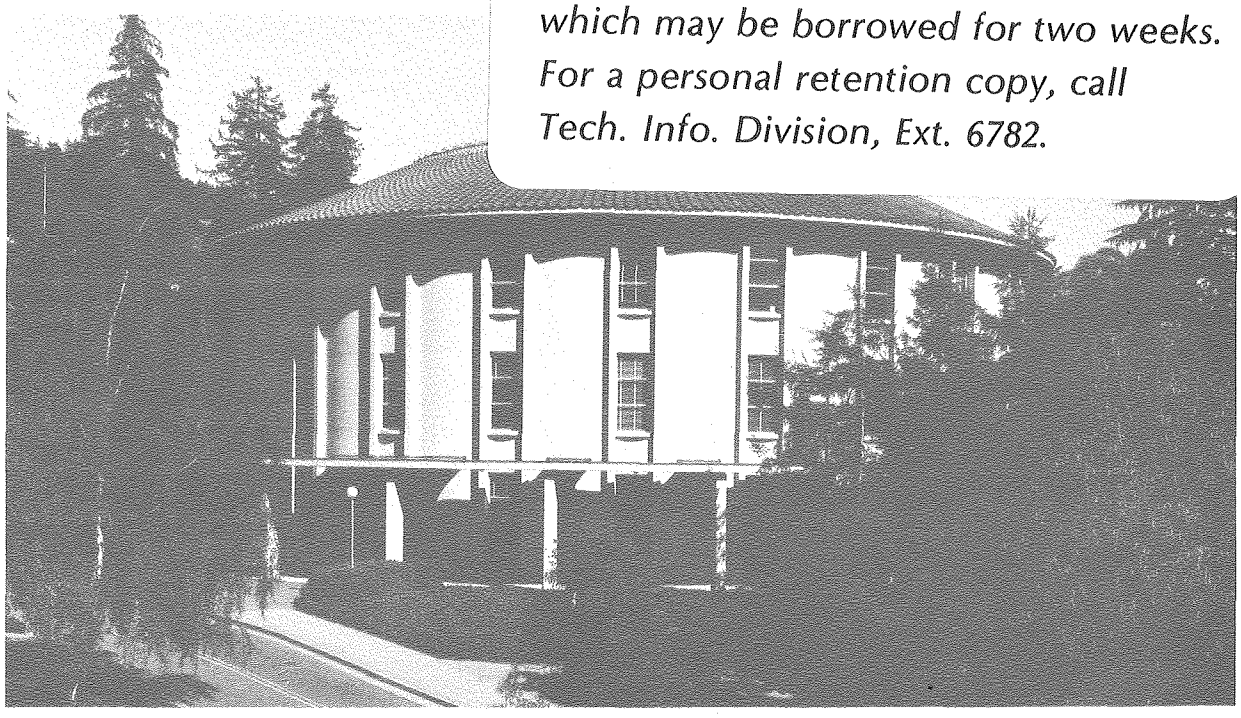

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INTRACELLULAR CONCENTRATIONS AND METABOLISM OF CARBON COMPOUNDS IN TOBACCO CALLUS CULTURES: EFFECTS OF LIGHT AND AUXIN[1]

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Abbreviations: NAA: naphthalene acetic acid; 2iP: 2-isopentenylaminopurine; PEP: P-enolpyruvate.

ABSTRACT

Callus cultures derived from pith tissues of Nicotiana tabacum were grown on two media either under continuous illumination or in complete darkness. The first medium limited greening ability of callus grown in the light (3.0 mg/l naphthalene acetic acid, NAA; 0.3 mg/l isopentenyl-aminopurine, 2iP; Murashige and Skoog salts and 2% sucrose). The second medium encouraged chlorophyll synthesis (greening) though not shoot formation (0.3 mg/l NAA; 0.3 mg/l 2iP). To measure intracellular concentrations, calli were grown for 15 days on standard media containing [U-^{14}C]sucrose. Many metabolite concentrations were proportional to the dry wt (as a fraction of fresh wt) of the calli: nearly doubling in light-grown cells and increasing 30 to 40% on low-auxin media. Glutamine concentrations (from 4 to 26 mM) very high probably due to NH_3 content of the media. Proline concentrations increased 20-fold in calli grown on low-auxin media in the light (green cells), possibly a stress response to high osmotic potentials in these cells. To analyze sucrose metabolism, callus cells were allowed to take up 0.2% (w/v) [U-^{14}C]sucrose for up to 90 min. In callus tissues and in pith sections from stems of tobacco plants, sucrose was primarily metabolized through invertase activity, producting equal amounts of labeled glucose and fructose. Respiration of $^{14}CO_2$ followed the labeling patterns of tricarboxylic acid cycle intermediates. Photorespiration activity was low.

Even though plant tissue cultures can be used as sources of new biochemical and genetic variation in plants, relatively few details are known about their chemical composition or the effects of physiological parameters on their composition. Isolated reports on metabolite concentrations can be found such as those for glutamine, alanine and malate (2), UDP-glucose (5) and the adenosine and nicotinamide nucleotides (4,5).

The effects of auxin on metabolite concentrations and metabolism of callus cultures havebeen indirectly characterized by Thorpe and colleagues in their studies on organogenesis (4,20,21). Effects of light on tissue cultures have been reported by many sources. For example, light decreased nicotine production in tobacco cultures (15), and altered metabolite patterns during CO_2 fixation in both heterotrophically and photoautotrophically grown tobacco cells (13).

Heterotrophically grown callus cultures of plants use sucrose as their primary source of energy and carbon skeletons. Most indications are that sucrose is primarily hydrolyzed by invertase action (6), however enzymatic analysis of callus cultures, especially during organogenesis, has suggested that sucrose synthase activity could play a major role in sucrose metabolism (21,22). Analysis of enzyme patterns has implicated a major role for the pentose phosphate pathway in the oxidation of glucose during organogenesis of tobacco callus culture (21).

The intracellular concentrations of carbon compounds which are reported here are intended as a general data set for plant callus cultures. The effects of light and auxin on the metabolic concentrations will be characterized. These concentrations, along with the observed metabolism of [U-^{14}C]sucrose by our callus cultures, will be compared with those ovserved by others using whole plant tissues or related tissue culture systems.

MATERIALS AND METHODS

Callus Cultures. Callus cultures were derived from the pith tissues of Nicotiana tabacum (var. Wisconsin 38) stems. Pith explants and calli were grown in petri dishes (100 x 15 mm) on a modified Linsmaier and Skoog (11) medium containing nutrient salts ("Murashige and Skoog Plant Salt Mixture", Flow Labs., Inglewood, CA), 100 mg/l inositol, 0.4 mg/l thiamine, 2% sucrose, and 1% agar, with 3.0 mg/l naphthalene acetic acid, NAA[2], and 0.3 mg/l 2-isopentenylaminopurine, 2iP. Calli were subcultured every 4 weeks. When subculturing prior to the experiments described below, the calli were transferred onto two media. The first medium was that described above and is referred to in the text as the "high-auxin" medium. The second, referred to as "low-auxin" medium, has one tenth as much NAA (0.3 mg/l). The callus cultures on both media were then separted into two sets and grown at 24 C, either in complete darkness or under continuous illumination of 100-120 $\mu E\ m^{-2}\ sec^{-1}$.

Growth studies of callus cultures were performed with cells which were chronologically identical. Dry wt determinations were made by heating replicate calli samples separately at 100 C for 24 h.

Intracellular Concentration Determinations. The experimental determinations of intracellular concentrations described in the text used callus cells which had been subcultured on high-auxin medium in the dark for 5 months prior to subculture onto either high- or low-auxin media and growth in the light or dark as described above. After 18 days the calli were transferred into 60 x 20 mm petri dishes and incubated under the same 4 conditions except that the media contained [U-^{14}C]sucrose (13.48 μCi/mg-atom C). The calli were then incubated for 15 days under these conditions to allow the ^{14}C derived from sucrose to equilibrate fully into

the carbon positions of the cellular metabolites.

Four samples from each growth condition were analyzed (2 callus samples from 2 different dishes). Only 3 samples of cultures grown in the dark on low-auxin medium were used. Each sample consisted of 1.0 g fresh wt of agar-free callus. The intercellular sucrose was removed by washing the cells twice with 5 ml of 0.2% (w/v) sucrose followed by three washes with 5 ml H_2O (allowing washes to sit for 10 sec before removal by vacuum through a Whatman #1 filter). The sample was then homogenized in 1 ml 80% (v/v)ethanol at 4 C and washed into conical centrifuge tubes with 1 ml 80% ethanol. The homogenate was centrifuged three times, the pellet being resuspended in 2 ml H_2O each time. Chloroform, 2 ml, was then added to the combined supernatants in a capped conical centrifuged tube and shaken. The aqueous phase was removed and its volume reduced to less than 2 ml by blowing N_2 gas over the surface. Aliquots of the insoluble pellet and the aqueous and chloroform extracts were used for determinations of radioactivity.

The aqueous phase was then passed through a cation exchange column (0.5 x 4 cm, Bio-Rad AG 50W-X8, hydrogen form, 200-400 mesh, Bio-Rad Labs, Richmond, CA) and further analyzed following a modification of the methods of Larsen et al (9). A water eluate containing the sugars, sugar phosphates, and organic acids was collected and the neutral and acidic amino acids were eluted with 4 ml of 1 N pyridine. The basic amino acids and strong bases were then eluted with 4 ml of 3 N NH_4OH. Aliquots of all the fractions were used for determining radioactivity. Their volumes were then decreased with a stream of N_2 gas.

The sugar and organic acid eluate was fractionated by two-dimensional descending paper chromatography, using high-pH phenol and butanol-propionic

acid solvents as described previously (17). The amino acid fractions were separated by paper chromatography as described by Larsen et al (9) using butanol: acetic acid: water (12:3:5 v/v) in the first dimension followed by butanol: methyl ethyl ketone: NH_4OH: H_2O (5:3:1:1 v/v) in the second dimension. After location of the labeled compouds by radioautography, the spots were cut out, soaked in 2.5 ml H_2O, and their radioactivity determined by scintillation counting with 15 ml of aqueous counting solvent.

The very high concentrations of glutamine and asparagine found in our callus cultures interferred with the chromatographic analysis of glycine, serine, alanine, and α-amino butyric acid. Therefore, portions of the neutral and acidic amino acid fractions were acid hydrolyzed with 3 N HCl at 100 C for 24 h in vacuo. The samples were then analyzed by paper chromatography and radioactivity determinations as above. The increases in glutamate and aspartate labeling were used to calculate the amount of glutamine and asparagine present, respectively.

To verify independently that the amino acids were metabolically saturated with ^{14}C-carbon during the 15 days on media containing [U-^{14}C]sucrose, the specific radioactivities of the amino acids were determined using [G-^{3}H]dansyl chloride as described by Larson et al (9). Because of the high concentrations of glutamine and asparagine present, a higher [G-^{3}H]dansyl chloride concentration, 24.4 mM (0.82 $\mu Ci\ \mu mol^{-1}$) was used. The amino acid dansyl derivatives were separated by thin layer chromatography (1), located with UV light, cut out, and their radioactivity determined by scintillation counting in 0.5 ml Protosol (New England Nuclear, Boston, MA), 2 drops of acetic acid, and 15 ml nonaqueous counting solvent.

Metabolism of sucrose. Tobacco callus cultures and pith sections from the stems of tobacco plants were supplied with [U-^{14}C]sucrose which was taken up and metabolized over periods ranging from 20 to 90 min in various experiments. In the experiment described in the text, the callus cells used were chronologically identical to those used in the intracellular concentration experiment decribed above. After 4 weeks of growth in the light or dark on either low- or high-auxin containing media, 1.0 g fresh wt of calli were carefully placed onto a Whatman #42 filter moistened with 0.3 ml of 0.2% (w/v) [U-^{14}C]sucrose (4.25 μCi/μg-atom C) which was fitted onto the bottom of a 5 ml Fernbach flask. A shortened, 1.5 ml plastic centrifuge tube containing a 1.7 x 2.0 cm Whatman #42 wick soaked with 50 μl of 3 N KOH was placed in the center of the flask to measure $^{14}CO_2$ release. The flask was then stoppered and slowly shaken for 45 or 90 min under room lights at 30 C. Duplicate samples for all conditions and time points were obtained. After the incubation period, the $^{14}CO_2$-containing wick was removed and placed in a vial containing 0.5 ml Protosol and 15 ml nonaqueous counting solvent. The cells were then quickly transferred and washed in cold sucrose and water, homogenized, fractionated, and analyzed as described above for the determination of intracellular metabolite concentrations.

RESULTS

Callus Cultures. Growth characteristics of the calli used in our studies are shown in Table I. Consistent values for the relative dry weights of callus cultures, calculated as a fraction of the fresh weights, were obtained when comparing calli with identical chronologies of explanting and subculturing. However, significant variation in the dry wt proportion was observed between calli differing in these parameters, even

Table I

when grown under identical conditions. In the calli studied for Table I, subculturing cells onto new high-auxin dishes caused a transient increase in their dry wt proporptions as measured 1, 2, and 3 weeks after subculturing. By four weeks after subculture, the dry wt values had reverted back to 25 mg dry wt g frest wt^{-1}. This transient increase was observed in both light- and dark-grown cultures.

When cells grown in the dark or high-auxin media were subcultured onto low-auxin media, dry wt proportions increased with time (Table I). The dry wt proportions of dark grown calli apparently stabilized by 3 weeks after subculturing, decreasing only slightly after four weeks. Light-grown callus cultures on low-auxin media (green cells) steadily increased their dry wt proportions through 4 weeks.

Increases in total dry wt (Table I) suggest that the calli doubling times ranged from 2 to 7 days depending on the growth conditions. Callus cultures grown in the dark on high-auxin medium commonly showed rapid increases in fresh and dry wt through the 4 weeks of culture (Table I). The largest increases in dry wt were observed in light-grown, low-auxin cultures (green cells).

Table II

The amount of labeling found in callus cultures grown for 2 weeks on [^{14}C]sucrose-containing media was approximately proportional to the cultures relative dry wt values (Table II). A direct estimation of the dry wt of the cultures from the labeling data can be made asssuming that each μg-atom ^{14}C-carbon is equivalent to one μmol of the general formula CH_2O (1 μg-atom = 30 μg dry wt). Callus which was dark-grown on high-auxin medium and had a total labeling of 227 μg-atoms C g fresh wt^{-1} (Table II) would therefore be equivalent to 6.8 mg dry wt g fresh wt^{-1}. This only accounts for about half the 15.2 mg dry wt g fresh wt^{-1} which was found in these

cultures (Table II). Calculations for calli grown under the other conditions also result in low estimates. This suggests that the 2-week growth of callus cultures with labeled sucrose did not completely label constituents with slow turnover rates (such as the cell walls, the largest cellular fraction). The lack of compete labeling on [^{14}C]sucrose-containing media could be overcome by initiating growth with small inoculi and allowing the calli to incorporate [^{14}C]sucrose for a complete sub-culturing cycle.

Intracellular Concentrations. The intracellular concentrations of 32 compounds found in calli are given in Table III. Concentration determinations were made by allowing the metabolite pools to equilibrate with radiolabeled carbon for 2 weeks. When callus cultures were put onto [U-^{14}C]sucrose-containing media and analyzed after 2,4,6, and 8 days for incorporation of label into metabolites, it was found that the ^{14}C incorporation into sugars, organic acids, and most amino acids had saturated after 4 days (data not shown). This implied that the carbon atoms of the metabolites had reached the same degree of labeling as that of the [^{14}C]sucrose supplied. Growth for 15 days on [U-^{14}C]sucrose-containing media was carried out in the experiment detailed here to assure that these pools saturated.

Table III

Tritiated dansyl chloride derivatives of the ^{14}C-amino acids were made to independently verify their specific radioactivities. All the amino acids analyzed had as high a specific radioactivity as that of the [^{14}C]sucrose supplied except for glutamate, glutamine, and asparagine. These three amino acids were from 40 to 85% "saturated" depending on the growth conditions. Accordingly, the values for intercellular concentrations of these amino acids (Table III) were derived by

quantitatively accounting for this lack of total labeling. It is likely that glutamate, glutamine and aspargine are primarily end-product and/or nitrogen-storage compounds in these cultures and therefore have very slow turnover rates.

The most dramatic difference in relative concentrations of metabolites between our callus cultures and plants was the very large glutamine concentrations in the callus, ranging from 20,800 to 130,000 ng-atoms g fresh wt^{-1} (or from 4 to 26 mM) (Table III). Asparagine concentrations in our cultures were also unusually large. Glutamine was the largest single fraction found in our analysis.

Many concentraion changes brought about by light or auxin (Table III) were proportional to the overall changes in the relative dry wt (Table II). However, many compounds followed patterns quite independent of changes in dry wt ratios. Sucrose concentrations were almost 10-fold higher in callus grown in the light on low-auxin media (green callus). Sucrose content was 48,200 ng-atom g fresh wt^{-1} (or 4 mM) in these cells (Table III). Maltose, glyerate and malate concentrations were also disporportionately large in green callus when compared to concentrations in callus grown under other conditions. Glucose and fructose concentrations responded identically to the various growth conditions. Their concentrations on a µmol g fresh wt^{-1} basis were larger than those of sucrose. Pentose concentrations (primarily ribose and xylose in tobacco plants, see 19) increased in calli grown on low-auxin containing media. UDP-glucose and pentose phosphate levels were too low to detect in our system which places their concentrations well below 100 ng-atoms g fresh wt^{-1}.

Proline concentrations increased more than 20-fold (to 5160 ng-atom g fresh wt^{-1}) in callus grown in the light on low-auxin media (Table III).

Under all other conditions proline concentrations were about 250 ng-atom g fresh wt^{-1}. Citrate concentrations were about 5-fold higher in dark-grown callus culture than in those grown in the light.

Table III also lists two compounds, P1 and P2, which chromatographed on paper in the region of 2- and 3-carbon phosphorylated compounds (such as 3-P-glycerate, P-enolpyruvate, P-glycolate, and P-serine; though chromatographic analyses have shown that the compounds are not any of the above acids). The two compounds are listed because of their dramatic dependence on auxin concentrations in the media. The concentrations of both compounds increased an average of 13-fold in tissue grown on high-auxin media whereas illumination during growth had almost no effect on their pool sizes.

Concentrations of β-alanine in calli were unaffected by changes in auxin concentration in the media but were almost 2-fold higher in dark-grown cultures. Short-term [U-^{14}C]sucrose incorporation and metabolism in our callus cultures (described below) did not produce detectable levels of labeled β-alanine, an atypical observation when compared to other amino acids with similar concentration ranges. This suggests that much of the β-alanine may be formed by decomposition of uridine and cytidine residues.

Sucrose Uptake and Metabolism. The uptake and subsequent metabolism of [U-^{14}C]sucrose by callus tissues was examined in several experiments. In the experiment described in this section two time points (45 and 90 min) were taken and duplicate samples for all times and conditions were obtained.

The uptake of sucrose, calculated from the total intracellular labeling of compounds in a sample, was greater in callus grown in the

Fig. 1

light. The pattern of sucrose uptake was similar to that of [^{14}C]sucrose metabolism shown in Fig. 1. Glucose and fructose labeling accounted for 55 to 82% of the metabolism of [^{14}C]sucrose (Fig 1). The metabolically active hexose pools in callus grown on low-auxin media were nearly equilibrated with ^{14}C after 45 min and were apparently smaller than the metabolically active hexose pools of high-auxin calli, which showed no signs of ^{14}C-saturation by 90 min.

As shown in Figure 1, glucose and fructose labeling was essentially equal at all time points. In addition, the percent of total metabolism accounted for in glucose and fructose labeling decreased with time in most experiments. When pith tissue sections from stems of tobacco plants were allowed to take up and metabolize [^{14}C]sucrose under identical conditions to those used for calli, glucose and fructose labeling was again equal and decreased with time (from 10 min) as a percent of total metabolism (data not shown). [^{14}C]UDP-glucose accounted for, at most, 0.08% of the labeled sucrose-metabolites found in both callus and pith section experiments. The labeling of UDP-glucose did not saturate during the experiments.

Only light-grown callus accumulated [^{14}C]sucrose internally (Fig. 1). This accumulation did not correlate with the intracellular sucrose concentrations observe (Table III) and is possibly indictive of a light-dependent sucrose loading into the vacuoles. Labeled maltose also accumulated in light-grown callus, though at only 1/100 the rate of [^{14}C]sucrose.

The labeling patterns of glucose-P and fructose-P (Fig. 1) did not follow the labeling patterns of the glucose and fructose from which the phosphorylated sugars were derived. Labeling of hexose monophosphates was significantly higher in dark-grown callus. The difference in the hexose

monophosphate labeling patterns compared to those of glucose and fructose suggested that the hexose kinases in light-grown cultures may have used substrates from small hexose pools separate from to the large metabolically active pools observed. Alternatively, light could alter hexose kinase's affinities toward substrates and/or products, or the availability of ATP.

The greater labeling in dark-grown cells observed in glucose-P and fructose-P (Fig. 1) was also observed with most of the subsequent metabolites, such as glycolysis and tricarboxylic acid cycle intermediates. The metabolically active hexose monophosphate pools were therefore probably used directly for further metabolism.

The rate of $^{14}CO_2$ release was greater by the latter time point (Fig. 2). A lag of $^{14}CO_2$ respiration was not unexpected in that the possible sources of CO_2 release (pyruvate, isocitrate, 2-oxoglutatrate, malate, oxalacetate, and glycine) are not primary products of [^{14}C]sucrose metabolism and would be labeled only subsequent to the primary metabolites such as the hexoses and hexose monphosphates. The increase in ^{14}C respiration with time could also be indicative of increased total repiration due to the wounding of callus tissues prior to the experiment.

Fig. 2

Of all the possible precursors of CO_2 respiration, malate and citrate had labeling patterns which most closely resembled the pattern of $^{14}CO_2$ release (Fig. 2). In callus cultures grown in the dark, the amount of label found in CO_2 malate and citrate during the second 45 min of [^{14}C]sucrose metabolism was 5 to 7 times larger than the relative labeling during the first 45 min (Fig. 2). In light-grown callus this ratio was about 2. In contrast to these similarities, Fig. 2 shows that glycine and serine labeling, indictive of photorepiratory $^{14}CO_2$ release, was comparatively linear with time.

CONCLUSIONS

Many of the metabolite concentrations observed in our callus cultures were similar to those reported with tobacco plants (14,19). Similar citrate (12,23) and proline (18) effects have been previously observed in whole plants. The observed increases in citrate levels in our dark-grown callus are in agreement with earlier observations using tobacco leaves (12,23). The large increase in proline in our green callus cells could be a stress response brought about by the high metabolite concentrations present in calli grown under these conditions. Proline concentrations are known to increase in many plants under various stress conditions (such as high salt or low water), possibly as a reliever of high osmotic potential in the cells (18). Increase in intracellular proline levels in response to osmotic stress in tomato tissue cultures have been recently reported (7).

Some of the metabolite concentrations observed in culture differed greatly from those seen in plants. Glutamine and, to a lesser extent, asparagine levels were extremely high in our callus. High glutamine concentrations in tobacco tissue cultures have been observed previously (2,10). Bergmann et al (20) attribute the large gluatmine concentraions to the presence of NH_3 in the media. Other culture systems (such as rice and sycamore) accumulate γ-aminobutyric acid in the presence of NH_3 (8). The content of γ-aminobutyric acid was not high in our cultures (data not shown). The most abundant free amino acid found by Noguchi and Tanaki in the leaves of tobacco plants was γ-aminobutyric acid (14).

Increases in extracellular sucrose have been reported to suppress chlorophyll synthesis in tissue cultures systems (16). The large intracellular sucrose concentrations of our green callus would therefore imply that the sucrose suppression of greening does not act by simply

raising the total intracellular sucrose pools.

Both our heterotrophically grown tobacco callus and the pith tissues from which they were derived primarily metabolize sucrose through invertase activity into glucose and fructose, which are then phosphorylated and further metabolized through glycolysis. Our use of [U-^{14}C]sucrose as a probe of primary carbohydrate metabolism adds a new approach to the studies of sucrose metabolism in tissue cultures. Our results lead to conclusions which differ from those of Thorpe and Meier (22), who indicated that sucrose synthase and UDP-glucose pyrophosphorylase could play major roles in sucrose metabolism. Their measurements were taken throughout the growth cycle, however,and therefore cover periods, such as the early exponential growth phase, which our current studies did not observe. Their conclusions were based on in vitro assays of various sucrose metabolizing enzymes. Though the presence of an enzyme activity makes a particular metabolic function possible, the quantitative importance of that function in metabolism in vivo need not be great. The presence of sucrose synthase and UDP-glucose pyrophosphorylase in tobacco callus tissues seems more likely to be related to sucrose synthesis rather than its metabolism.

The ratio of [^{14}C]glucose to [^{14}C]fructose found during [U-^{14}C]sucrose metabolism in both pith and callus tissues was always near unity which is indicative of invertase activity. Such labeling would not be expected if sucrose was primarily broken down to glucose and fructose via UDP-glucose, glucose-1-P, and glucose-6-P by the enzymes sucrose synthase, UDP-glucose pyrophosphorylase, phosphoglucomutase, and glucose-1-phosphatase. The percent of ^{14}C-metabolism which was found in glucose and fructose declined slightly from 10 to 50 min in both pith and callus tissues, supporting the hypothesis that the hexoses were primary products of metabolism.

The observed accumulations of labeled glucose and fructose with time could result from cell wall invertase activity hydrolyzing extracellular sucrose into glucose and fructose in the free space of the callus tissues. This is not likely, however, since callus tissues were washed extensively before analysis of ^{14}C-incorporation. In addition, [^{14}C]sucrose concentrations in dark-grown cells were lower than those expected if free space constituents were also being measured.

UDP-glucose labeling increased with time throughout the assaying periods in both pith and callus tissues and showed no apparent saturation of its metabolicllly active pool. These observations are consistent with a relatively unimportant role for sucrose synthase activity. If the low UDP-glucose labeling was due to a quick conversion of newly labeled UDP-glucose to glucose-1-P via UDP-glucose pyrophosphorylase, then the ratio of [^{14}C]glucose-1-P to [^{14}C]UDP-glucose would be expected to be approximately constant at early time points. This was not observed; instead hexose-monophosphate labeling often saturated after 20 min of metabolism (e.g., Fig. 1).

The similarities in the time course of $^{14}CO_2$ release and ^{14}C labeling of malate and citrate suggest that the major source of $^{14}CO_2$ respiration in our cultures was the tricarboxylic acid cycle. Labeling of glycine and serine indicted that photorespiration was relatively inactive. However, glycine and serine labeling were higher in light-grown callus relative to the labeling patterns of other metabolites (excepting glucose and fructose). This suggests that photorespiration was induced to some extent in callus cultures grown in the light. Low rates of photorespiration in heterotrophically grown tobacco callus cultures have been reported previously (3). High rates of photorespiration would be expected in

photoautotrophically grown callus light grown cells, or by growth on media which supports photosynthetic differentiation, such as a low-auxin media.

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TABLE I. *Growth Characteristics of Callus Cultures*

Callus grown on 3.0 mg/l NAA-containing media in the dark were subcultured onto either low- or high-auxin containing media and grown under either continuous illumination or complete darkness, as indicated, for up to 4 weeks. The initial dry wt of the calli was 24.4 ± 0.7 mg dry wt·g fresh wt^{-1}. Determinations of fresh and dry weights of three or four calli were used for each value shown. Averages of these determinations are given with their standard deviation of the mean.

	Dark Grown		Light Grown	
Growth Period in weeks	3.0 mg/l NAA	0.3 mg/l NAA	3.0 mg/l NAA	0.3 mg/l NAA
	mg dry wt·g fresh wt^{-1}			
1	30.4±3.3	25.2±1.9	31.3±1.5	36.4±4.3
2	28.2±1.0	30.4±1.3	35.3±1.6	44.8±1.5
3	26.4±0.5	31.4±2.1	33.7±1.3	47.9±2.1
4	25.1±2.7	27.8±0.6	25.6±1.4	55.7±2.3
	dry wt of callus·dry wt of $inoculum^{-1}$			
1	1.81±0.21	1.26±0.48	0.80±0.17	2.32± 0.85
2	2.94±1.14	8.07±2.60	3.08±0.73	7.27± 2.34
3	4.26±0.93	17.3 ±1.8	6.78±3.16	26.6 ± 7.7
4	16.7 ±2.3	14.6 ±0.9	19.9 ±8.2	44.2 ±14.0

Table II. *Comparison of Dry Weights of Callus Cultures with Long-Term ^{14}C-Labelling Patterns.*

The calli used for determinations of dry and fresh wt were identical to those used for pool size determinations. Weight values shown are the averages of determinations from two calli. The variation between these measurements ranged from 1% up to 20%. The experimental procedure for pool size determinations using calli grown on [U-^{14}C]sucrose-containing media are described under "Materials and Methods." Values are shown with their standard deviation of the mean.

	Dark Grown		Light Grown	
	3.0 mg/l NAA	0.3 mg/l NAA	3.0 mg/l NAA	0.3 mg/l NAA
mg dry wt·g fresh wt^{-1}	15.2	19.7	27.5	40.4
Dry wt relative to dark-grown, high auxin callus	1.00	1.30	1.81	2.66
Pool Sizes From 2-Week [^{14}C]Sucrose Uptake		µg-atom C·g fresh wt^{-1}		
Water solubles	109.0 ± 8.0	149.0 ±17.0	174.0 ± 13	364 ±17.0
Chloroform Solubles	3.76± 0.16	3.81± 0.10	7.15±0.52	8.88± 0.67
Insolubles	114.0 ± 5.0	144.0 ±18.0	189.0 ±11.0	202 ± 8.0
Total	227.0 ±11.0	297.0 ±20.0	370.0 ±18.0	575 ±24.0
Total pool sizes relative to dark-grown, high-auxin callus	1.00	1.31	1.63	2.53

Table III. *Intracellular Concentration of Carbon Compounds: Effects of Light and Auxin*

Callus cultures grown in the dark on media containing 0.3 mg/l 2iP and 3.0 mg/l NAA were subcultured onto two media, 0.3 mg/l 2iP and 3.0 mg/l NAA (high auxin) or 0.3 mg/l 2iP and 0.3 mg/l NAA (low auxin), and grown in the light or dark for about 2 weeks before being transfered to identical media containing [U-^{14}C]sucrose for 15 days. Concentrations were analyzed as described under Materials and Methods. Values represent averages of four separate determinations from two different petri plates ± SE. To calculate μmol amounts, divide by number of carbon atoms.

	Intracellular Concentrations			
	Dark Grown		Light Grown	
	3.0 mg/l NAA	0.3 mg/l NAA	3.0 mg/l NAA	0.3 mg/l NAA
	ng-atoms C/g fresh wt			
Sucrose	6,160 ± 2,070	2,520 ± 620	3,880 ± 600	48,200 ± 5,200
Glucose	15,100 ± 4,600	11,000 ± 2,400	20,200 ± 2,600	48,900 ± 3,600
Fructose	17,500 ± 4,800	14,100 ± 3,100	35,400 ± 4,300	53,900 ± 2,800
Pentoses	326 ± 84	609 ± 69	349 ± 36	557 ± 141
Maltose	123 ± 35	127 ± 6	232 ± 42	1,270 ± 201
Glucose-P	153 ± 29	110 ± 3	91 ± 16	170 ± 20
Fructose-P	168 ± 41	93 ± 9	114 ± 22	146 ± 6

Table III. *Intracellular concentration of Carbon Compounds: Effects of Light and Auxin* (cont'd)

	Intracellular Concentrations			
	Dark Grown		Light Grown	
	3.0 mg/l NAA	0.3 mg/l NAA	3.0 mg/l NAA	0.3 mg/l NAA
	ng-atoms C/g fresh wt			
Glycerate	112 ± 19	122 ± 16	238 ± 18	852 ± 310
Malate	6,780 ± 330	7,370 ± 510	5,070 ± 550	22,000 ± 1,100
Citrate	5,780 ± 710	4,630 ± 180	371 ± 64	906 ± 14
PEP	137 ± 27	109 ± 18	188 ± 25	267 ± 34
P-1	1,100 ± 180	73 ± 10	912 ± 256	139 ± 52
P-2	2,840 ± 200	173 ± 18	5,480 ± 1,030	350 ± 96
Asp	410 ± 110	1,090 ± 80	947 ± 51	2,980 ± 250
Glu[a]	3,020 ± 630	6,460 ± 230	3,920 ± 200	6,720 ± 280
Asp[a]	1,710 ± 200	2,860 ± 560	5,830 ± 660	21,100 ± 5,700
Gln[a]	20,800 ± 9,600	65,300 ± 5,100	58,400 ± 3,600	130,000 ±11,000
Ala	402 ± 63	1,480 ± 470	417 ± 25	699 ± 202
Gly	205 ± 11	292 ± 30	428 ± 15	514 ± 61
Ser	< 150	270 ± 74	497 ± 57	733 ± 2

Table III. *Intracellular concentration of Carbon Compounds: Effects of Light and Auxin* (cont'd)

	Intracellular Concentrations			
	Dark Grown		Light Grown	
	3.0 mg/l NAA	0.3 mg/l NAA	3.0 mg/l NAA	0.3 mg/l NAA
	ng-atoms C/g fresh wt			
Pro	187 ± 28	351 ± 32	208 ± 19	5,160 ± 300
Val	121 ± 20	260 ± 29	401 ± 25	379 ± 24
Tyr	80 ± 9	202 ± 9	397 ± 17	419 ± 25
Phe	188 ± 37	619 ± 35	1,340 ± 70	1,280 ± 100
Try	136 ± 27	96 ± 7	174 ± 12	158 ± 69
Ile	72 ± 10	105 ± 10	342 ± 30	190 ± 24
Leu	73 ± 9	183 ± 21	277 ± 18	688 ± 72
Thr	101 ± 18	265 ± 21	502 ± 17	640 ± 19
Arg	99 ± 30	205 ± 12	258 ± 22	341 ± 40
His	212 ± 53	585 ± 41	559 ± 41	774 ± 45
Lys	118 ± 29	258 ± 11	265 ± 16	394 ± 44
β-alanine	223 ± 37	221 ± 13	136 ± 23	136 ± 27

Table III. *Intracellular Concentration of Carbon Compounds: Effects of Light and Auxin* (cont'd)

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[a]Glu, asn, and gln were not saturated in ^{14}C-carbon after 15 days as determined by specific radioactivity checks using [^{3}H]dansyl chloride derivatives of the amino acids (see Materials and Methods). The concentrations of glu, asn, and gln have been corrected for this by dividing by appropriate saturation factors.

LEGENDS TO FIGURES

Fig. 1. Effects of light and auxin on primary metabolism of [U-^{14}C]sucrose. Callus cultures grown in the dark on media containing 0.3 mg/l 2iP and 3.0 mg/l NAA were subcultured onto two media, 0.3 mg/l 2iP and 3.0 mg/l NAA (high auxin) or 0.3 mg/l 2iP and 0.3 mg/l NAA (low-auxin), and grown in the light or dark for 4 weeks. Upper portions of the callus were then allowed to take up and metabolize 0.2% (w/v) [U-^{14}C]sucrose for 45 or 90 min as described under Materials and Methods. Values shown are the averages of two determinations during one experiment. Other experiments have given similar results. Variation from average was about 20% or less. One 90 min sample of light-grown, high auxin callus was discarded. Dark-grown, high-auxin (▲——▲); dark-grown, low-auxin (●- - -●); light-grown, high-auxin (△——△); light-grown, low-auxin (○- - -○). Sucrose metabolism is calculated as the total intracellular recovery of labeled compounds and $^{14}CO_2$ release excluding [^{14}C]sucrose.

Fig. 2 Effect of light and auxin on $^{14}CO_2$ respiration and the labeling of possible sources of respiratory CO_2 release. Experimental conditions were the same as those in Fig. 1 and described under Materials and Methods. Values shown are averages of two determinations during one experiment. Other experiments have given similar results. Variation from average was about 20% or less except for $^{14}CO_2$ values which showed variation of 40% or less. One 90 min sample of light-grown, high-auxin callus was discarded. Dark-grown, high-auxin (▲——▲); dark-grown, low-auxin (●- - - -·●), light-grown, high-auxin (△——△); light-grown, low-auxin (○- - -○).

Fig. 1

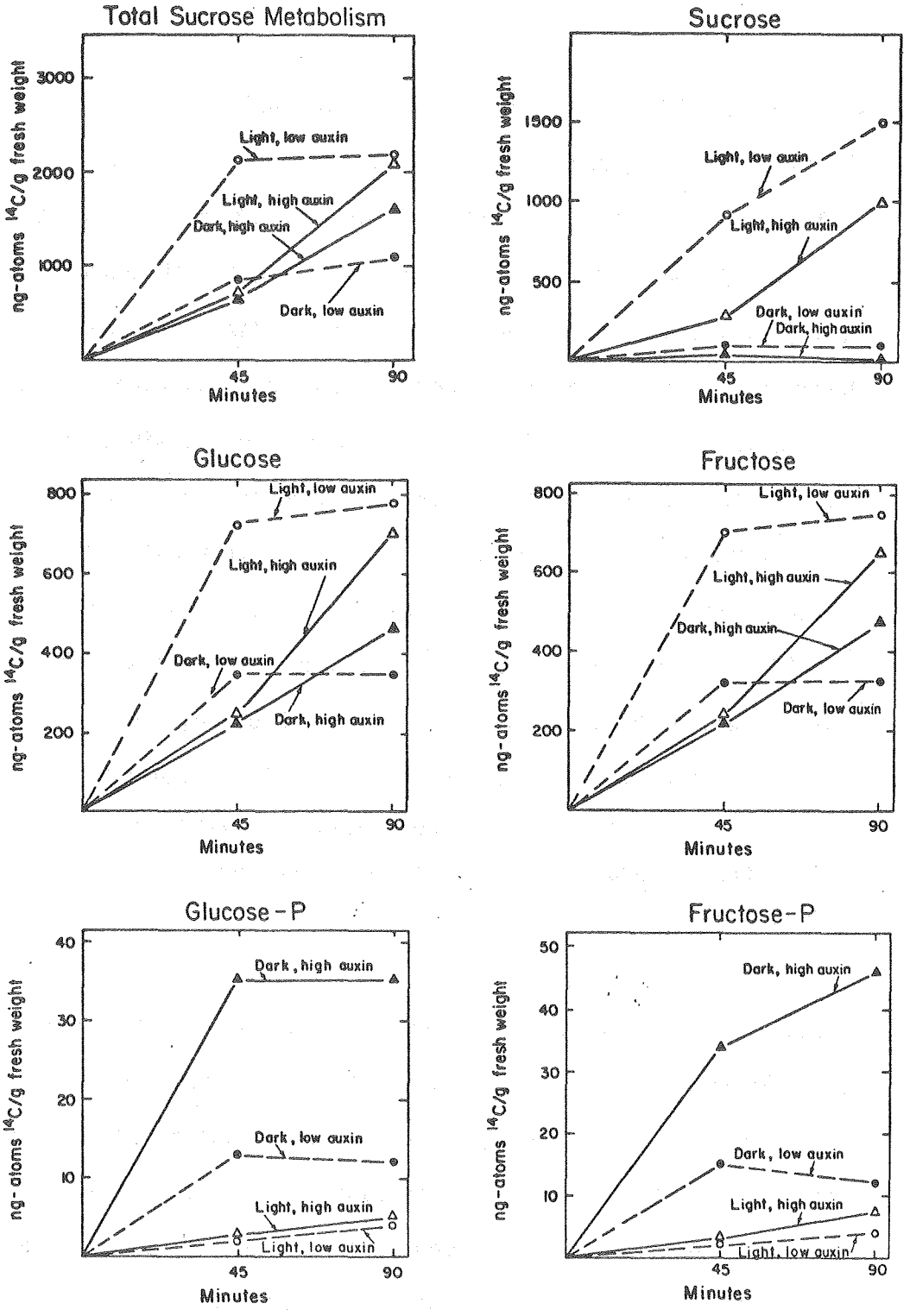

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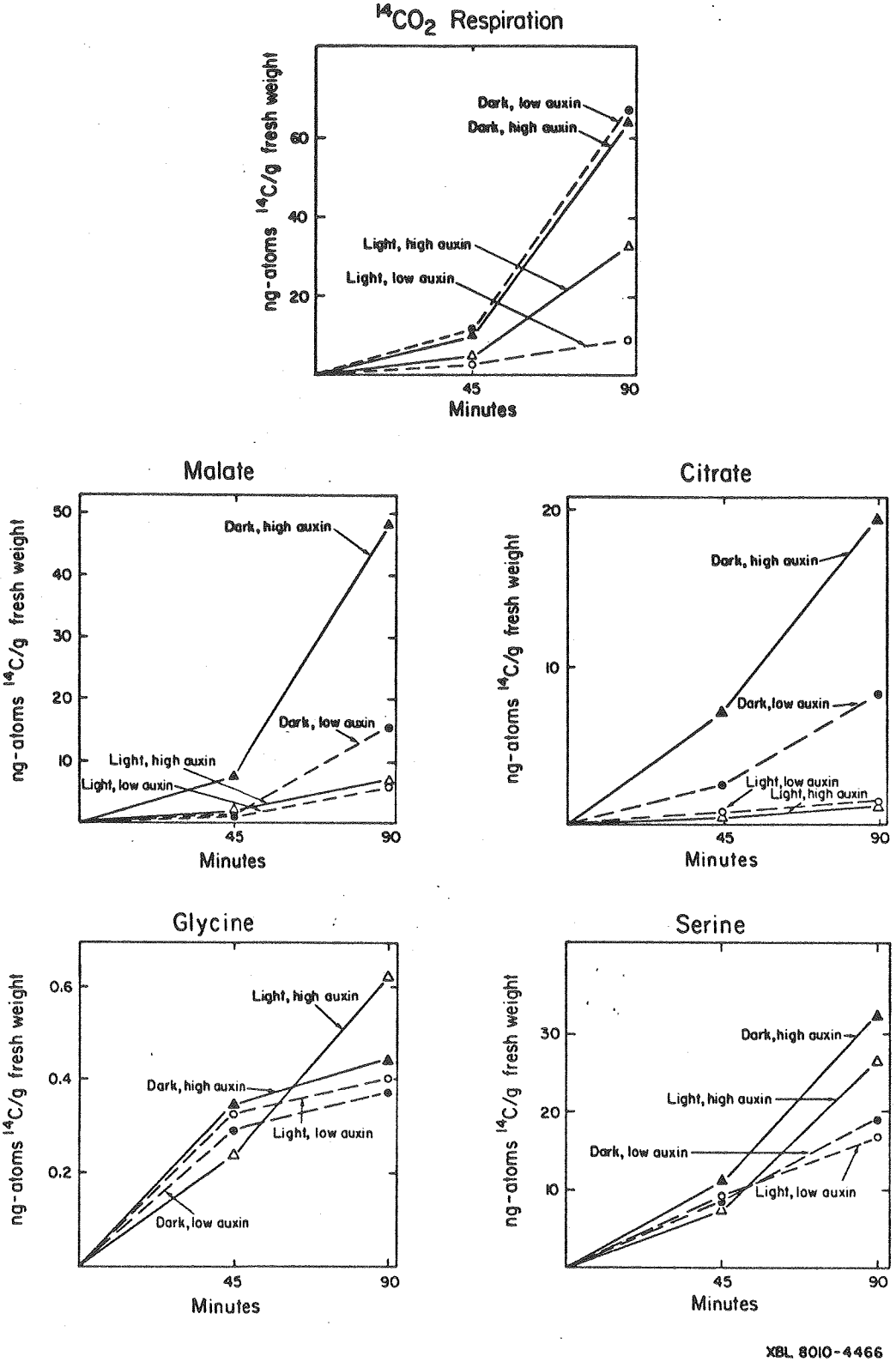
Fig. 2


$^{14}CO_2$ Respiration
ng-atoms ^{14}C/g fresh weight
Dark, low auxin
Dark, high auxin
Light, high auxin
Light, low auxin
Minutes
Malate
Citrate
Glycine
Serine
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